

WHAT IS CLAIMED IS:

1. A method for activating a lymphocyte, comprising aggregating three or more antigens expressed by the lymphocyte, and activating the lymphocyte.

5 2. The method of Claim 1 in which the lymphocyte is a T cell.

3. The method of Claim 2 in which the T cell expresses CD4.

10 4. The method of Claim 2 in which the three or more antigens are selected from a combination of CD2, CD3, CD4, CD5, CD6, CD8, CD18, CD25, CD27, CD28, CD40, CD43, CD45, CD45RA, CD45RO, CDw137, CDW150, CD152, CD154, ICOS, TCR alpha, TCR beta, TCR delta, TCR gamma, and a cytokine receptor.

15 5. The method of Claim 4 in which the antigens are aggregated by a single multispecific molecule.

6. The method of Claim 4 in which the antigens are aggregated by one or more antibodies or an antigen-binding derivative thereof.

20 7. The method of Claim 6 in which the antibody contains only heavy chains or an antigen-binding derivative thereof.

8. The method of Claim 7 in which the antigen-binding derivatives are V_{HH} .

25 9. The method of Claim 4 in which the antigens are aggregated by peptides.

10. The method of Claim 9 in which the peptides are derived from antibody complementarity determining regions.

30 11. The method of Claim 4 in which the antigens are aggregated by their corresponding ligands.

12. The method of Claim 6 in which the antibodies or antigen-binding derivatives are immobilized on a solid surface.

5 13. The method of Claim 12 in which the antibodies or antigen-binding derivatives are conjugated to a particulate substrate.

14. The method of Claim 12 in which the antibodies or antigen-binding derivatives are arranged in a sequential order.

10 15. The method of Claim 2 in which the T cell is activated to proliferate.

16. The method of Claim 2 in which the T cell is activated to produce cytokines.

15 17. The method of Claim 2 in which the T cell is activated to alter its expression of cell surface antigens.

18. The method of Claim 2 in which the T cell is activated to alter its expression of cytokines.

19. The method of Claim 2 in which the T cell is activated to undergo apoptosis.

25 20. The method of Claim 2 in which the lymphocyte is a B cell.

21. The method of Claim 20 in which the three or more antigens are selected from a combination of surface Ig, CD18, CD19, CD20, CD21, CD22, CD23, CD40, CD45, CD80, CD86, B7.3 and ICAM 1.

30 22. The method of Claim 20 in which the B cell is activated to proliferate.

23. The method of Claim 20 in which the B cell is activated to undergo apoptosis.

5 24. A multispecific protein comprising binding sites specific for three or more antigens expressed on the surface of a lymphocyte.

25. The multispecific protein of Claim 24 which activates a T cell.

10 26. The multispecific protein of Claim 24 which inhibits activation of a T cell.

27. The multispecific protein of Claim 24 which activates a B cell.

15 28. The multispecific protein of Claim 24 which inhibits activation of a B cell.

29. The multispecific protein of Claim 24 which comprises V_{HH} domains.

20 30. The multispecific protein of Claim 24 which comprises complementarity-determining regions.

31. A pharmaceutical composition, comprising the multispecific protein of Claim 24.

25 32. A bispecific protein comprising binding sites specific for two antigens expressed on the surface of a lymphocyte, and inhibits activation of the lymphocyte.

33. The bispecific protein of Claim 32 in which the lymphocyte is a T cell.

30 34. The bispecific protein of Claim 32 in which the lymphocyte is a B cell.

35. The bispecific protein of Claim 32 which comprises V_{HH} regions.
36. The bispecific protein of Claim 32 which comprises complementarity determining regions.
- 5 37. A pharmaceutical composition comprising the bispecific protein of Claim 32.
- 10 38. An isolated heavy chain-only antibody which binds to a cell surface antigen or an antigen-binding derivative thereof.
39. The antigen-binding derivative of Claim 38 which is V_{HH} .
- 15 40. A method for isolating a B cell expressing heavy chain-only antibodies, comprising isolating B cells from a cell mixture, said B cells express CD40 and do not express an immunoglobulin light chain.
- 20 41. A cDNA library comprising polynucleotides which encode llama V_{HH} regions.
42. The cDNA library of Claim 41 in which the polynucleotides encode heavy chain-only antibodies.
- 25 43. An isolated polypeptide, comprising an amino acid sequence selecting from the group consisting of SEQ ID NOS: 1-9.
44. A modified phage display vector, as depicted in Figure 14.
- 30 45. A method of cloning and expressing llama V_{HH} that binds human lymphocyte surface antigens using the vector of Claim 44.

46. A method of llamaizing a heavy chain variable region coding sequence comprising

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(a) annealing two complementary oligonucleotides at about the midpoint of said heavy chain variable region coding sequence; and

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(b) extending the annealed oligonucleotides by overlapping single stranded primers by polymerase chain reactions; wherein the oligonucleotides encode an amino acid residue at position 11, 37, 44, 45 or 47 that is not present in said heavy chain variable region coding sequence.

47. A fusion protein comprising a llama constant domain of CH1, hinge, CH2, CH3 or a combination thereof and a heterologous non-llama polypeptide.

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48. A peptide comprising the amino acid sequence selected from the group consisting of SEQ ID NOS:61-63, 69-71, 72-74, 75 and 80.

49. A soluble human CD3 heterodimeric polypeptide.